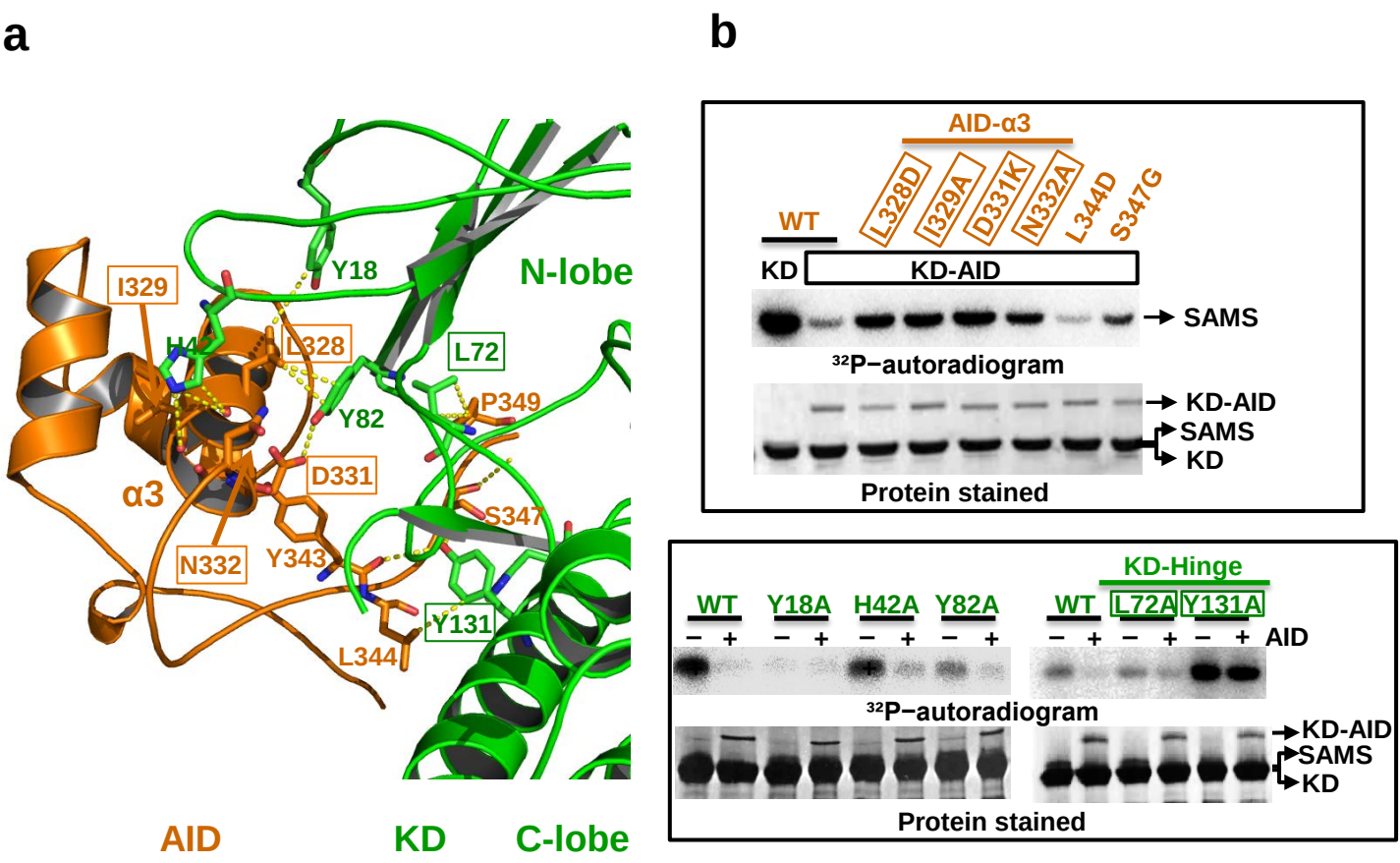

Supplementary Figure 6. KD and AID form a monomeric cis-complex at moderate protein concentrations. (a) Close-up of the *trans* interface in the crystal structure of the human KD–AID fragment. The interface is formed by residues L328, I329, D331, and N332 from the conserved α3 helix of the AID packing against Y18, H42, and Y82 from the N-lobe of the KD as well as by L344, A345, S347, and P349 from the C-terminal loop of the AID packing against L72 and Y131 from the KD hinge. Note that in yeast the corresponding interface residues from the AID α3 helix (yeast L341, L342, E344, N345) and hinge (yeast L88, Y146) directly interact with each other[21]. **(b)** Mutational analysis of the KD–AID interface. Activities of wildtype and mutant KD–AID (for AID mutations) and KD (for KD and AID mutations) proteins were determined by [γ-32P]-ATP kinase assays. Replacement of the interface residues from the AID α3 and the hinge relieved AID repression of kinase activity, while replacement of α3-interacting residues on the KD (Y18A, H42A, Y82A) or hinge-interacting residues on the AID loop (L344D, S347G) did not, or only partially, relieved AID repression. This indicates that the interfaces of the human and yeast KD–AID complexes involve the same interactions (α3 and hinge) and that in the structure of the human complex the interacting surfaces have moved relative to each other due to crystal packing. In the human KD–AID model shown in Fig. 4d and 4e, the AID has therefore been repositioned by structural alignment with the yeast AID.